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Comparison of Inhibitory Activities of Various Antiretroviral Agents against Particle-Derived and Recombinant Human Immunodeficiency Virus Type 1 Reverse Transcriptases

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Several known antiretroviral agents were tested for their ability to inhibit the activities of human immunodeficiency virus type 1 reverse transcriptase purified from virions or from a recombinant *Escherichia coli* strain. The recombinant reverse transcriptase, a polypeptide of 66 kilodaltons, showed inhibition profiles indistinguishable from those of the virion-derived enzyme with all tested compounds, except for suramin and two dextran sulfates. These were more inhibitory to the recombinant enzyme, presumably because the *E. coli*-derived enzyme was more highly purified. The relative ease with which large quantities of recombinant enzyme can be prepared should facilitate the large-scale screening and identification of new potential inhibitors of the human immunodeficiency virus type 1 reverse transcriptase.

Reverse transcriptases (RT) are virus-coded enzymes that are specific and essential for the replication of retroviruses such as the human immunodeficiency virus type 1 (HIV-1). The enzyme catalyzes the transcription of viral RNA into DNA. No cellular homolog of viral RT has been discovered, except those derived from endogenous retroviruses and retrotransposons. For this reason, RT is considered one of the most prominent targets for antiretroviral chemotherapy (13, 17). Di Marzo Veronese and colleagues have reported the purification and characterization of HIV-1 RT by using an immunoaffinity chromatographic procedure based on the mouse hybridoma, which recognizes the p66/51 proteins (4). Several groups have expressed HIV-1 RT in bacteria (2, 9, 11, 12; R. T. D'Aguila and W. C. Summers, 28th Intersci. Conf. Antimicrob. Agents Chemother. abstr. no. 1302, 1988). Large amounts of purified HIV-1 RT can be isolated from recombinant bacteria for extensive structural and biochemical studies. Recently, Hizi et al. (9) were able to construct an HIV-1 RT expression clone that, when inserted into *Escherichia coli*, induced the synthesis of large amounts of a protein with an apparent molecular size of 66 kilodaltons (kDa) that differed from HIV-1 RT only in that it had two additional amino-terminal amino acids. The RT activities expressed by this polypeptide and that isolated from HIV-1 virions showed similar inhibition profiles with ddTTP and ddGTP.

In this study the inhibitory effect of several known kinase-dependent (8, 17, 19, 21; C. K. Chu, R. F. Schinazi, M. K. Ahn, G. V. Ullas, and Z. P. Gu, J. Med. Chem., in press) and -independent (3, 5, 14–16, 20) HIV-1 RT inhibitors were compared against partially purified virus particle-derived RT and purified RT derived from the *E. coli* clone described by Hizi et al. (9). The four nucleotides analogs tested were 3′-azido-dTTP, 3′-azido-ddUTP, 5-ethyl-ddUTP, and ddTTP. The six nonnucleosides evaluated were phosphonoformate (sodium salt), phosphonoacetate (sodium salt), 21-tungsto-9-antimoniate (ammonium salt), suramin (sodium salt), and two different dextran sulfates of ~8 kDa (DS-8,000; sodium salt) and ~1.340 kDa (DS-1,340; potassium salt). The nucleotides were prepared by the method of Yoshikawa et al. (22). ddTTP was obtained from U.S. Biochemical Corp. (Cleveland, Ohio). 21-Tungsto-9-antimoniate, phosphonoformate and phosphonoacetate, suramin, DS-8,000, and DS-1,340 were obtained from C. Hill (Emory University), Astra Alab (Södertälje, Sweden), M. Le Maitre (Rhône-Poulenc, Vitry-sur-Seine, France), ICN Biochemicals Co. (Cleveland, Ohio), and Calbiochem (San Diego, Calif.), respectively. Virus-derived RT was isolated from detergent-disrupted virions obtained from the cell-free supernatant of HIV-1 (strain LAV)-infected, phytohemagglutinin-stimulated human peripheral blood mononuclear cells. The enzyme was purified by passing the extract through two ion-exchange chromatography columns as described previously (8). The enzyme was characterized as HIV-1 RT based on its cation, salt, pH, and template requirements. The purification of RT from *E. coli* will be reported elsewhere (manuscript in preparation). A standard reaction mixture was used to evaluate the inhibitory effect of the drugs as described previously (6) and in footnote *a* of Table 1. The median effective concentration (EC_{50}) was determined by using at least four different concentrations of each compound. These values were derived from the computer-generated median effect plot of the dose-effect data as described previously (1, 2, 18).

The EC_{50}s obtained with the different compounds with recombinant and virion-associated RT are compared in Table 1. For the recombinant HIV-1 RT, the compounds listed in their increasing order of inhibitory potencies were as follows: 3′-azido-dTTP ≥ 3′-azido-ddUTP ≥ ddTTP > 5-ethyl-ddUTP ≥ phosphonoformate > DS-8,000 > suramin > DS-1,340 > 21-tungsto-9-antimoniate ddUTP ≫ phosphonoacetate. The ranking of the potency of the compounds and their EC_{50}s was similar between the two enzymes with the exception of suramin and the two dextran sulfates, DS-8,000 and DS-1,340. The latter two compounds were about 10-fold less inhibitory to the particle-derived RT than to the recom-

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TABLE 1. Comparison of concentrations of various antiviral compounds producing a 50% reduction of the activities of virus particle-derived and recombinant HIV-1 reverse transcriptases[a]

Compound	Particle-derived RT		Recombinant RT	
	EC_{50} (μM) ± SD	Slope ± SD	EC_{50} (μM) ± SD	Slope ± SD
Nucleotides				
3'-Azido-dTTP	0.0049 ± 0.0024	0.80 ± 0.12	0.0070 ± 0.0041	1.00 ± 0.24
3'-Azido-ddUTP	0.0096 ± 0.0024	0.93 ± 0.14	0.0098 ± 0.0050	1.02 ± 0.17
5-Ethyl-ddUTP	0.116 ± 0.0047	0.85 ± 0.01	0.108 ± 0.064	1.09 ± 0.14
ddTTP	0.0088	1.08	0.0081	1.20
Nonnucleotides				
PFA	0.14 ± 0.03	0.94 ± 0.12	0.20 ± 0.06	0.94 ± 0.23
PAA	>500		>500	
Suramin	17.8	2.53	8.57	1.67
HPA-23	31.9 ± 10.0	5.16 ± 1.03	26.2 ± 2.42	1.46 ± 0.15
DS-8,000 (+BSA)	37.7 ± 7.8	1.28 ± 0.23	4.48 ± 1.10	1.49 ± 0.31
DS-8,000 (−BSA)	3.8	0.65	0.44 ± 0.23	0.78 ± 0.07
DS-1,340 (+BSA)	1,332 ± 363	1.56 ± 0.02	257 ± 11.3	1.70 ± 0.11
DS-1,340 (−BSA)	170	0.78	17.0 ± 6.1	0.98 ± 0.03

[a] EC_{50}s were derived from at least two different experiments; when more than two experiments were performed, the standard deviation was provided. EC_{50}s and slopes were obtained by using median effect plots (1, 2). In these studies, the correlation coefficients for the plots were ≥0.98. The RT assays for all the compounds except the dextran sulfates were performed in the presence of 100 μg of BSA per ml. Unhibited enzyme activity gave 33,930 ± 540 cpm and 914,800 ± 2,010 cpm (mean values ± standard deviations of eight determinations) for the particle-derived and recombinant RT, respectively. This was equivalent to 4.8 and 128 pmol of TMP incorporated into newly synthesized DNA after 60 min of incubation of a 100-μl reaction mixture containing 100 mM Tris chloride (pH 8.0), 50 mM KCl, 2 mM $MgCl_2$, 5 mM dithiothreitol, 3.1 μg of poly(rA)$_n$ · oligo(T)$_{12-18}$, and 1 μM [^{3}H]TTP. The RT activity assayed without BSA resulted in 16,700 ± 442 cpm (2.3 pmol of TMP) and 414,860 ± 22,257 cpm (58 pmol of TMP) for the particle-derived and recombinant enzyme, respectively. The addition of 100 μg of BSA per ml stimulated the RT activities by 100 to 120%. PFA, Phosphonoformate; PAA, phosphonoacetate; HPA-23, 21-tungsto-9-antimoniate.

binant RT (Table 1). This difference may be attributed to the higher amount of non-RT proteins present in the virion-derived enzyme material that may bind to the dextran sulfates and, thus, lower the amount of RT-reactive inhibitor molecules. To further illustrate the nonspecific protein-binding effect of dextran sulfates, we compared the effect of DS-8,000 on the particle-derived HIV-1 RT activity assayed with and without the addition of 100 μg of bovine serum albumin (BSA; fraction V; Sigma Chemical Co., St. Louis, Mo.) per ml. The inhibition profiles (Fig. 1) clearly illustrate the HIV-1 RT-protective effect of BSA. The EC_{50} shifted from 3.8 μM in the absence of BSA to about 30 μM in the presence of 100 μg of BSA per ml, an eightfold decrease in the inhibitory activity of DS-8,000. Similar experiments performed with the recombinant RT also demonstrated a reduction of the inhibitory potential of the DS-1,340 and

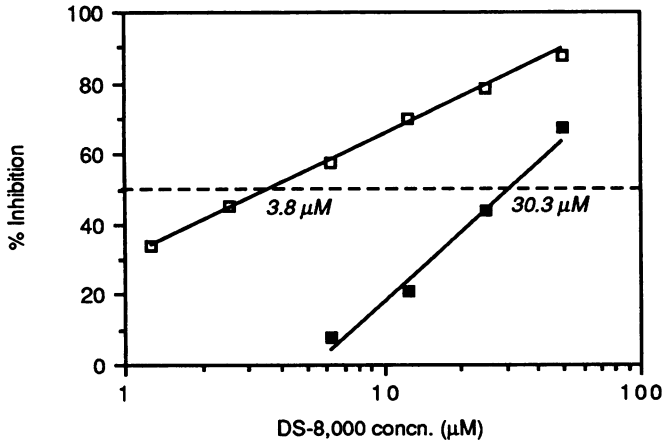

FIG. 1. Effect of dextran sulfate (DS-8,000) on particle-derived HIV-1 RT activity assayed in the presence (■) and absence (□) of 100 μg of BSA per ml.

DS-8,000 by 10 to 15 times (Table 1). With the exception of suramin and the dextran sulfates, the results indicated that several RT inhibitors with different structures and potencies produced similar inhibitory profiles with the virion-derived and recombinant enzymes. Dextran sulfates have been reported to inhibit HIV-1 RT, virus replication, and syncytium formation (10, 14, 20). In agreement with Mitsuya et al. (14), DS-8,000 was a potent inhibitor of HIV-1 RT activity when assayed in the presence of low amounts of protein (Table 1). The nonspecific protein-binding capacity of the dextran sulfates may also explain the failure of Ito et al. (10) to observe an inhibition of HIV-1 RT in crude extracts of disrupted virions at 25 μM (125 μg/ml) with a dextran sulfate with a molecular size of approximately 5 kDa. The addition of BSA (100 μg/ml) produced a significant stimulatory effect (100 to 120%) of both the particle-derived and recombinant RT activities. However, this finding is not in agreement with the observations of Mitsuya et al. (14) and may be explained by differences in the assay conditions and characteristics of the enzyme used.

In conclusion, our observations indicate that the recombinant 66-kDa HIV-1 RT expressed in and isolated from *E. coli* was appropriately inhibited by a variety of antiretroviral agents. This enzyme can be used in automated cell-free screening systems for potential anti-RT compounds that do not depend on being biotransformed into activated forms by other enzymes. Nucleosides, which are kinase dependent, must be converted to corresponding 5'-triphosphates before testing in this screening system. Because large amounts of the recombinant HIV-1 RT can be produced and purified to yield an enzyme with a high specific activity, low levels of protein may be used in each assay. Consequently, more nonspecific protein-binding inhibitors will be identified when the purified recombinant HIV-1 RT is used instead of the particle-derived material. This problem can be overcome by including BSA in the reaction mixture. Compounds identified as potent inhibitors of the recombinant HIV-1 RT should

also be evaluated against partially purified host-cell DNA polymerases to determine their degree of selectivity.

The recombinant HIV-1 RT has several advantages over virion-derived RT for evaluating potential antiretroviral agents: ease of preparation in large scale at a low cost; high specific activity; high purity; and, most important, the absence of infectious virus particles, which reduce the risks involved in working with concentrated HIV-1 infected material. The availability of a large quantity of HIV-1 RT with a high stability would also make it a suitable candidate as a reference or standard for RT assays.

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